

Commonwealth of Virginia

Harmful Algal Bloom Response Plan

INTRODUCTION

Phytoplankton are microscopic algae that are common members of freshwater and marine habitats. They represent a major source of food and oxygen for many of the inhabitants present in lakes, rivers, estuaries, and oceans. Among the several thousand species of phytoplankton that exist worldwide, approximately 70 to 80 of these are known toxin producers. These toxins are potentially harmful to humans as well as to birds, fish, and other inhabitants of aquatic habitats.

Usually, toxin-producing algae are present in low concentrations and pose no environmental or human health threat. However, with certain environmental conditions, toxin-producing algae may proliferate to form dense concentrations of cells on water surfaces referred to as “harmful algal blooms” (HABs). HABs have been documented in the coastal waters of both the eastern and western United States, as well as other coastal areas throughout the world. In Virginia, these HABs may occur from early spring through the fall months. To date there are 16 potential toxin producing species that have been recorded in Virginia waters and the lower Chesapeake Bay, with the possibility of other toxic species becoming established.

The heat-stable toxins produced by HABs may accumulate in certain shellfish and finfish and cause illness when ingested. Although shellfish and finfish may become toxic and cause human illness in the absence of a bloom, illness is usually associated with the presence of a bloom. The most widely recognized human illnesses caused by HABs are:

- Amnesic shellfish poisoning (ASP)
- Ciguatera fish poisoning (CFP)
- Diarrhetic shellfish poisoning (DSP)
- Neurotoxic shellfish poisoning (NSP)

- Paralytic shellfish poisoning (PSP)

Most of the harmful algae related to these illnesses produce neurotoxins that cause a range of neurological and gastrointestinal symptoms depending on the toxin produced. In addition, inhalation of certain aerosolized toxins associated with HABs has been implicated as the cause of respiratory irritation in humans.

One of the more recently identified HABs in Chesapeake Bay estuaries and other locations on the eastern coast of the United States is *Pfiesteria*. This HAB may be linked to human illness characterized by fatigue, headache, respiratory irritation, skin lesions, disorientation, memory loss, and impairment of cognitive function. Some researchers have proposed that *Pfiesteria* is associated with fish kills as well as ulcerative lesions on fish skin. The possibility that *Pfiesteria* produces a toxin that may cause disease in humans and fish is under investigation.

Although the occurrence of HABs has been rare in Virginia's coastal waters, the occurrence of HABs worldwide appears to be increasing. The reason for the increase is likely due to many factors including climatic changes, anomalous weather events, transport of nonindigenous marine species through the ballast water of ships, and pollution of coastal waters. Improvement in detection of harmful algae and HABs may also contribute to their perceived increase. Because of the potential for HABs to occur in Virginia, a state with significant marine and estuarine waters, it is imperative that a comprehensive plan exists which describes and enables surveillance for and identification of HABs and possible health effects from HABs in order to protect the health of both humans and marine animals.

VIRGINIA HAB TASK FORCE

HABs are increasingly common in estuarine and coastal waters, both nationally and internationally, and are recognized as a growing threat to coastal living resources, economies and public health. The cause(s) of HABs and their increasing prevalence are likely varied and not well understood, but there is growing concern that they are associated with human activities. For example, increased nutrient inputs (primarily forms of nitrogen and phosphorus) from land to estuarine and coastal waters are suspected as an important factor. Because of the potential risk that HABs may pose to human health, natural resources and environmental quality, there is an increased urgency within various federal and

state agencies with responsibility for resource management, environmental protection and public health to support monitoring and research programs in relevant areas. During the summer of 1997, the presence of lesions on finfish and a modest fish kill, involving mostly menhaden, suggested that there might be a harmful algal bloom (HAB) species in Virginia's estuarine waters. From 1998 through 2003, Virginia's response to potential *Pfiesteria* events, fish lesion events and HAB events was coordinated by the Virginia *Pfiesteria* Task Force (now known as the Virginia HAB Task Force). The Virginia HAB Task Force will continue to facilitate the Commonwealth's response to HAB events by sharing and disseminating information and coordinating resources.

HAB Task Force Participants

The task force comprises members from five agencies and institutions: Virginia Department of Health (VDH), Virginia Institute of Marine Science (VIMS), Virginia Department of Environmental Quality (VDEQ), Old Dominion University (ODU) and the Virginia Marine Resources Commission (VMRC). In addition, there are a number of auxiliary agencies and academic institutions that share a direct or indirect role in task force efforts. They are the Department of Game and Inland Fisheries (VDGIF), Department of Conservation and Recreation (DCR), the Chesapeake Bay Local Assistance Department (CBLAD), the Marine Products Board (VMPB), Department of Agriculture and Consumer Services (VDACS), Virginia Commonwealth University (VCU) and Virginia Polytechnic Institute and State University (VPISU).

MONITORING AND RESPONSE TO HABs

Accurate assessment of HABs and associated impacts on Virginia's natural resources requires a monitoring program for HABs and their impacts through an integrated plan for a rapid and effective response to HAB-related events, then acquiring the necessary equipment, resources and expertise to implement the plan. There is a need to know the environmental influences (biotic and abiotic) which maintain and promote viable HAB species in Virginia estuaries. Currently, it is suspected that altered water quality, particularly from nutrient enrichment, may be a determinant of HAB abundance. However, direct evidence for the role of nutrients in stimulating HABs is inconclusive, and it is possible that mitigating environmental influences will differ among water areas. The VDH, VDEQ, and member

institutions will conduct monitoring and research to determine which environmental factors (e.g., nutrients, organic carbon, temperature, salinity) are consistent with HAB occurrences, then prioritize Virginia waters that have the potential of HABs occurring in large numbers.

Virginia has developed and implemented a multi-agency effort for response, monitoring and research on HAB organisms. This effort includes extensive participation by ODU and the VIMS, both of which have been active participants in Virginia's Chesapeake Bay Program. VDH, VDEQ, ODU and VIMS are all actively involved in HAB response, monitoring and research.

A seven-day per week, 24 hour per day HAB response capability is a joint effort between VMRC, VDH-DSS and VDEQ, with laboratory support by VIMS and ODU. VDEQ will also complement the VDH HAB program with an extensive network of stations for HAB and water quality sampling. A total of 20 stations in small estuaries on the eastern and western shores of the bay and in the major rivers of the Bay will be monitored for standard water quality parameters, HABs, chlorophyll and a full range of nutrients.

HAB Response Operational Plan

VDEQ has developed an operational plan for VDEQ staff response to suspected HABs and tidal fish kills. The plan establishes an agency HAB response program from May 1 through October 31 of each year. In addition, the plan outlines the initial, as well as follow up sampling protocols.

All suspected HABs or fish kills are referred to an initial HAB responder during the "HAB Season" from May through October. Each regional office ensures a HAB response through the regional pollution response program. If a name and number is available, the first step is to call the person who reported the fish kill or algae bloom to get first hand information.

If the event is in brackish or salt water (east of I-95), involves discolored water (red, brown, mahogany, or green), fish acting erratically, or fish kills, then it should be treated as a possible HAB event.

Once the responder has determined that the event should be investigated as a possible HAB event, the responder gathers response equipment and proceeds to the site. If a boat is necessary for access and one is not available through normal VDEQ means, the responder will contact VMRC (800-541-4646). The responder will arrange to meet VMRC at a mutually agreed boat launch as close as possible to the event.

The initial HAB responder will always take a complete package of HAB response equipment and supplies. This package of equipment and supplies will be staged and ready to go. If a site investigation confirms a possible HAB event, the responder will contact VIMS and ODU to arrange for sample delivery and to notify them of the event. The responder will also contact the VDH HAB contact and VDEQ management for initiation of the HAB communication protocol. Next, the responder will determine the extent of the alga bloom or fish kill and take appropriate samples for verification of the presence of HABs.

If the responder or VDEQ management feels it necessary, a follow-up response will be conducted. One staff will be identified to serve as the boat crew along with the initial responder. The follow-up response crew will deliver samples to the appropriate agencies. Every effort should be made to perform a follow-up response the next morning. The follow-up response will be for the collection of HAB organism samples, chlorophyll, water quality samples, and sediment.

HAB Monitoring

Twenty fixed stations determined by VDH, VDEQ, VIMS, and ODU will be sampled by VDEQ monthly from May to November. Samples will be collected for water quality parameters, genetic molecular analysis, and HAB/phytoplankton identification. Water quality samples will be sent to DCLS for analysis, genetic molecular samples to VIMS, and HAB/phytoplankton samples to ODU.

HUMAN DISEASE SURVEILLANCE FOR HEALTH EFFECTS FROM HABs

Health Effects

Health concerns center on direct exposure to HAB toxins in water related activities or professions, and illnesses associated with eating contaminated shellfish or fish. If it were determined that health effects result from exposure to these events, then appropriate investigations would consider the following.

- a) What are the health effects?
- b) How are they manifested?
- c) What is the pathogenesis?
- d) What are the risk factors for developing health effects?
- e) How can exposure or health effects be prevented?
- f) How can health effects be treated?
- g) How can exposure be measured?
- h) How can illness be definitively diagnosed?
- i) Is there a dose response?
- j) Which HABs are responsible?

HUMAN DISEASE SURVEILLANCE - Passive Surveillance

Increasing Awareness

The VDH Division of Zoonotic and Environmental Epidemiology (DZEE) will develop information on health effects from HABs and distribute it to medical care providers and local health departments via the Virginia Epidemiology Bulletin, the VDH website, and other venues (e.g. meetings, conference calls). DZEE will make information on HABs available to the public via brochures and the VDH website.

Reporting

Physicians will be urged to report suspected HAB health effects to their local health departments who will in turn notify DZEE. The HAB/*Pfiesteria* toll free line (888-238-6154) will be monitored daily during normal workdays for reports of suspected HAB related illness from the public.

Follow up

Local health departments and DZEE will complete the surveillance form supplied by CDC for each suspected case and will provide guidance to physicians on diagnostic testing and case management. DZEE will maintain a database of all reports, review the data for indications of increased risk, and supply the necessary data to CDC.

HUMAN DISEASE SURVEILLANCE - Active Surveillance and Outbreak Investigation**Triggers for initiating active surveillance**

Confirmed HAB due to an organism known to cause human illness

Cluster of human or other mammal illnesses associated with a recent HAB.

Active surveillance activities to be conducted by VDH district, regional and/or central office epidemiologists and other staff

Review records in local hospitals and medical practices

Utilize media to notify public to report cases, if necessary

Develop line list of potential cases, establish database with demographic, exposure and clinical information, summarize and analyze data.

Outbreak investigations will be conducted by VDH district, regional and central office epidemiologists and other staff in accordance with standard epidemiologic methods for data collection, analyses, report writing, summary findings, and recommendations.

Reports and Confidentiality

Summary reports of surveillance and outbreak investigations will be provided to all interested parties. Patient confidentiality will be protected and no personal identifying information will be released.

HAB COMMUNICATION SEQUENCE

The following outline should be followed with all due speed. Depending on seriousness of event, conference calls or face-to-face meetings can be scheduled.

1. Report of HAB or related health event received by any of agencies on attached HAB Communications List.
2. Immediately refer reporter or report directly as follows:
 - a. Health event: VDH
 - b. Fish kill or algal bloom: VDEQ
 - c. Fish with lesions: VIMS
3. Rapidly distribute a summary of any credible report via an email distribution list to Virginia contacts on HAB Communications List.
 - a. Notify federal agencies and other states if potential for multistate impact or media attention.
 - b. Include summary of knowledge to date and brief outline of plans for investigation.
4. Rapidly initiate investigation. Samples will be collected for water quality conditions, phytoplankton analysis (preserved and unpreserved samples), and genetic molecular analyses. Additional sample requirements (i.e., fish and shellfish samples) will be determined during the event.
5. Rapidly report initial findings to Virginia contacts on HAB Communications List and others as needed.
 - a. Include samples taken, time of delivery to which laboratory and estimated time of test results.
 - b. Provide suggested plans for further investigation and/or control.
6. Rapidly report laboratory results to Virginia contacts on HAB Communications List and others as needed.
7. Distribute final report to all interested parties in a timely manner.

HARMFUL ALGAL BLOOM PUBLIC INFORMATION PROCEDURES**Notification of Public and Media**

The Public Relations Coordinator (PRC) for the VDH Office of Epidemiology will be notified promptly of any HAB related events that may impact public health or cause public concern and will coordinate the development and distribution of public awareness messages with all agencies represented on the HAB Task Force. VDH will rely on the technical expertise of ODU and VIMS in preparing all such releases to the public and media.

VDH central office and local health departments will notify the public and regional media about an event, which can include a fish kill, algal bloom, or other water-related condition that may involve HABs and have an impact upon public health or may cause significant public concern. The PRC will coordinate with VDH Regional Public Information Officers (PIOs) to develop media announcements outlining the location of the HAB and clearly defining any prevention messages, including possible fish consumption advisories and swimming restrictions, to protect public health. This information will be made available on the VDH website as well.

In the event of a fish kill, algal bloom or other HAB-related situation that does not impact human health, but may be the source of public concern, VDH will provide information to the public about the situation via media release and/or posting on the VDH website depending upon the significance of the event. Announcements of events not impacting human health will be coordinated with all agencies represented on the HAB Task Force.

If a HAB related event requires the closure of a waterway to protect public health, the Commissioner of Health will order the closure and involved state agencies and the media will be notified. VMRC or DGIF will enforce the closure on their respective waterways. VDH will supply signs, which will be clearly posted to alert the public of the risks associated with contact with the water in that area. DGIF, VMRC and/or VDH local health departments will post the signs.

VDH will develop informative flyers on HABs which will include directions for individuals who may have been exposed to HABs during an event. These materials will be distributed to local health departments and other agencies.

VDH will share all media alerts, printed materials and other public information messages with involved agencies and will collaborate on message development in the event of a HAB related event.

VIRGINIA DEPARTMENT OF ENVIRONMENTAL QUALITY
HARMFUL ALGAL BLOOM (HAB) RESPONSE AND MONITORING
WORKPLAN FOR 2004

Virginia has developed and implemented a multi-agency effort for response, monitoring and research on HAB organisms. This effort includes extensive participation by ODU and the VIMS, both of which have been active participants in Virginia's Chesapeake Bay Program. A seven day per week, 24 hour per day HAB response capability is a joint effort between VMRC, VDH-DSS and DEQ, with laboratory support by VIMS and ODU. DEQ will also complement the VDH HAB program with an extensive network of stations for HAB and water quality sampling. A total of 20 stations in small estuaries on the eastern and western shores of the bay and in the major rivers of the Bay will be monitored for standard water quality parameters, HABs, chlorophyll and a full range of nutrients.

HAB RESPONSE OPERATIONAL PLAN

DEQ has developed an operational plan for DEQ staff response to suspected HABs and tidal fish kills. The plan establishes an agency HAB response program from May 1 through October 31 of each year. In addition, the plan outlines the initial, as well as follow up, sampling protocols.

All suspected HABs or fish kills are referred to an initial responder during the "HAB Season" from May through October. Each regional office shall ensure a HAB response through the regional pollution response program. If a name and number is available, the first step is to call the person who reported the fish kill or algae bloom to get first hand information.

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Once the HAB responder has determined that the event should be investigated as a possible HAB event, the responder gathers response equipment and proceeds to the site. If a boat is necessary for access and one is not available through normal DEQ means, the responder will contact VMRC (800-541-4646). The responder will arrange to meet VMRC at a mutually agreed boat launch as close as possible to the event.

The initial HAB responder will always take a complete package of HAB response equipment and supplies. This package of equipment and supplies will be staged and ready to go. If a site investigation confirms a possible HAB event, the responder will contact VIMS and ODU to arrange for sample delivery and to notify them of the event. The responder will also contact the VDH HAB contact and DEQ management for initiation of the HAB communication protocol. Next, the responder will determine the extent of the alga bloom or fish kill and take appropriate samples for verification of the presence of HABs.

If the responder or DEQ management feels it necessary, DEQ will activate a follow-up response. One staff will be identified to serve as the boat crew along with the initial responder. The follow-up response crew will deliver samples to the appropriate agencies. Every effort should be made to perform a follow-up response the next morning. The follow-up response will be for the collection of HAB organism samples, chlorophyll, water quality samples, and sediment.

Response Teams

DEQ has two response teams, from the Piedmont Regional Office (PRO), and the Tidewater Regional Office (TRO). Each team has an initial responder trained in all aspects of HAB response including personal safety.

Equipment and Funds

- Each regional office has an adequate ability to purchase supplies and support at the local scene,

especially for sustained incidents.

- **Sampling Equipment** - An adequate supply of sampling equipment is on hand in quantities sufficient to handle 30 days of continuous sampling.
- **Safety Equipment** - All safety equipment is available at each regional office.
- **Boat Fuel** - Fuel will have to be purchased from a commercial service station or marina after hours and on weekends and in situations where the time required to travel to a State facility would adversely impact the investigation.
- **Tools** - Tools for minor repairs of boats, trailers, sampling equipment will be brought on scene. These are currently available at the regional offices.
- **Ice and Coolers** - DEQ regional offices should have on hand sufficient ice and coolers for the investigation.

FIELD SAMPLING DURING RESPONSE TO HAB RELATED EVENTS

The following sampling protocols are designed for initial and follow-up response to HABs and fish kills.

Initial Response Water Quality and HAB Monitoring

It is important to arrive and sample while the HAB and/or fish kill still in progress. Because of the critical nature of the initial response, a protocol has been developed for conducting investigations when available staff may be limited. A DEQ responder, using vessels provided by DEQ, VMRC, VDH-DSS, or VDGIF, can arrive on site and collect the basic samples needed.

Some common relationships associated with HABs and HAB associated fish kills are:

1. Oxygen levels may be super-saturated or depressed.
2. Water may be discolored red, brown, mahogany or green.
3. Live fish may exhibit erratic movements.

First Response Equipment Kits: The following sampling equipment should be maintained in field kits to minimize response time.

1. Quart cubitainers (20)
2. 250 ml wide mouth HPDE bottle (10)
3. 2 liter brown Nalgene bottles (5)
4. Chlorophyll filtration kit
5. Lugol's solution (25 ml)**
6. Drop bottle with H₂SO₄
7. Lab Tags (30)
8. Lab Sheets (10)
9. Chain of custody sheets (5)
10. Discrete sampling bottle (Labline, Alpha, Kemmerer, etc.)
11. Neoprene gloves
12. Appropriate meters for field parameters (pH, DO, Temperature, Salinity)
13. Secchi disk
14. GPS
15. Field notebook/pens
16. Cellular phone and contact list

17. Fish Kill Manual
18. Binoculars
19. Ponar dredge
20. Stainless steel pan
21. Stainless steel scoop
22. Nautical charts
23. Water filtration kits provided by Reece laboratory at VIMS to include:
 - disposable filtering unit with Nuclepore filter in place
 - 2 funnel adapters, and 1 (#8) rubber stoppers with 1/2" hole
 - side-arm flask
 - vacuum pump or hand-held pump
 - disposable plastic coliform 120 ml water collection bottles
 - disposable forceps
 - 1.5 ml microcentrifuge tube containing 180ul lysis buffer+20ul proteinase K solution (to be replaced by tubes with fresh solution every 6-8 wks, if not used for sampling).

****Lugol's Solution:**

20 g Potassium iodide (KI)
10 g Iodine crystals
200 ml distilled water containing 20 ml glacial acetic acid

Field Observations All field measurements and observations should be recorded in a bound and numbered notebook.

1. Note any discoloration of the water.
2. Note any erratic movements or behavior by the fish.
3. Note size, species, and number of dead fish.
4. Observe fish for any unusual marks such as lesions, net marks, hook marks, abrasions, or parasites. Note the location on the fish and describe these marks.
5. Record tide, weather conditions, and any other pertinent information.
6. Record sampling coordinates using a GPS.

Field and Water Quality Parameters

Measure the following field parameters:

Dissolved oxygen
Temperature
Conductivity
Salinity
pH
Secchi Depth

Take surface samples for conventional water quality parameters (NME7), nutrients (NUT4 and TNUTL), and chlorophyll (FCHLR) using procedures in the current DEQ Water Quality Monitoring SOP Manual. Bottom samples should be taken if the depth is greater than 2 meters. Deliver or ship samples to DCLS.

Fish Enumeration

Follow the procedures described in the American Fisheries Society's Fish Kill Counting Guidelines (Appendix F of the 1997 DEQ Fish Kill Investigation Guidance Manual) for determining fish counts.

Fish Samples

1. Use the following guidelines in collecting fish for analysis.
2. Collect only distressed or dying fish.
3. Place fish in plastic bags and keep on ice (Do not freeze or preserve with formalin). Include an ID tag with date, time, location, collector, and phone number.
4. Deliver fish as soon as possible, preferably the same day, to Dr. Wolfgang Vogelbein (804-684-7261) or his associates at the Virginia Institute of Marine Science (VIMS). Call first to make arrangements. If Dr. Vogelbein or his associates cannot be reached, keep the fish on ice until the next business day.
5. Include all field information and a map or description of the location with GPS coordinates.

Water Filtration for Genetic Molecular Analyses (VIMS)

1. Take water sample in water collection bottle. If unable to filter at site, place collection bottle on ice.
2. Attach funnel adapter and stopper to disposable test filter funnel. Stopper may not be necessary if using a branched filter flask. Place funnel adapter into neck of flask and rest filter funnel on rim. Filter 100 mls of water, adding 20 mls at a time, if necessary, due to turbidity of water.
3. Use disposable forceps to remove 3 um nuclepore filter from filtration apparatus. Fold filter in half and then in half again using the forceps and place in microcentrifuge tube containing buffer solution. With forceps, push filter into bottom of tube so that it is submersed in the liquid. Dispose of test filter funnel, collection bottle, and forceps. Do not dispose of funnel adapter, or stopper.
4. Label microcentrifuge tube (site, date, mls) and store upright in box provided. Samples should remain at room temperature.
5. Deliver samples and Chain of Custody field data sheet to Dr. Reece's Laboratory as soon as possible at VIMS, Chesapeake Bay Hall -North 119. Kim Reece – 804-864-7407, Gail Scott or Bill Jones 804-684-7235/7873

Water and Sediment Samples at Monitoring Stations to be Used for Detecting the Presence of HABs (ODU)

1. Collect one Lugols preserved and one unpreserved water sample (1000 ml) at both the surface (<1m) and above the bottom (within 1 ft from the bottom) if the depth is greater than 2 meters using a hose connected to a pump, or with a collection (Alpha) bottle lowered to the lower depth. If the water depth is less than two meters, collect only surface water samples at a depth of one meter.
2. Collect a grab sample of the superficial sediment layer with a Petit Ponar dredge in the center of the HAB, fish kill or lesion event. The top 2-4 cm should be transferred to a collection jar. No preservative is added. The sample should be kept in the shade, and not iced. Approximately 250 ml of sediment should be collected.
3. The unpreserved water samples should be kept away from the sun, in a cooler, with little jostling, and separated from the preserved samples and Lugols solution. The water and sediment containers should be labeled with station number, collection date and time, collector, and depth taken.
4. The Chain of Custody field data sheet and water and sediment samples should be delivered to the ODU Plankton Analysis Laboratory within 24 hours. Laboratory contacts are: Dr. Harold Marshall (757-683-4204), and the laboratory manager, Todd Egerton (757-683-4994)

Follow-up Water Quality Monitoring

Follow-up monitoring is required to substantiate and supplement data collected during the initial response. The monitoring design, frequency, and duration will be determined after consultation with HAB Task Force members.

FIELD SAMPLING AT FIXED STATION NETWORK (2004 Monitoring Season)

Stations

Twenty fixed stations determined by VDH, DEQ, VIMS, And ODU will be sampled monthly from May to November 2004. Samples will be collected for water quality parameters, genetic molecular analysis, and HAB/phytoplankton identification. Water quality samples will be sent to DCLS for analysis, genetic molecular samples to VIMS, and HAB/phytoplankton samples to ODU. The station locations and map are shown below.

Water Quality Parameters and Frequency

Water samples should be taken in conjunction with the HAB samples. At the 8 fixed CBP monitoring stations, collect standard CBP parametric samples for filtered and particulate nutrients, solids, particulate carbon, chlorophyll, and bacteria. At the 12 Small Coastal and Eastern Shore fixed stations collect nutrients, solids, chlorophyll, and bacteria. Fixed stations will be sampled monthly from May to November 2004. For a HAB response event, collect nutrients, solids, chlorophyll, bacteria with the Small Coastal/Eastern Shore parameters, plus any additional parameters that may aid in identifying the cause of the event. Samples will be collected in accordance with DEQ Water Quality Monitoring SOPs.

Specific water sample parameter lists are below:

Tributary Station

Temperature (Profile)
pH (Profile)
Salinity (Profile)
Conductivity (Profile)
D.O. (Profile)
Light Attenuation (Licor Profile)
Secchi
Turbidity (Lab Measurement)
Total Suspended Solids
Volatile Suspended Solids
Fixed Suspended Solids
Ammonia, Dissolved
Nitrite, Dissolved
Nitrate, Dissolved
Nitrite & Nitrate, Dissolved
Orthophosphorus
Silica, Dissolved
Total Dissolved Nitrogen
Total Dissolved Phosphorus
Particulate Phosphorus
Particulate Carbon
Particulate Nitrogen
Chlorophyll A, Uncorrected
Chlorophyll A, Corrected

Small Coastal/Eastern Shore/HAB response

Temperature
pH
Salinity
Conductivity
D.O.
Turbidity (Lab Measurement)
Total Suspended Solids
Volatile Suspended Solids
Fixed Suspended Solids
Total Kjeldahl Nitrogen
Total Phosphorus
Total Orthophosphorus
Total Ammonia
Total Nitrite
Total Nitrate
Chlorophyll A, Uncorrected
Chlorophyll A, Corrected
Chlorophyll B (Trichromatic)
Chlorophyll C (Trichromatic)
Pheophytin A
Enterococci (SW)
E. Coli (FW)

1 qt cube lugols preserved algae sample

Chlorophyll B (Trichromatic)

Chlorophyll C (Trichromatic)

Pheophytin A

Fecal Coliform

E. Coli

Enterococci

1 qt cube lugols preserved algae sample

1 filtered genetic molecular probe sample

1 sediment sample (once per monitoring season)

1 filtered genetic molecular probe sample

1 sediment sample (once per monitoring season)

Note: DCLS Group Codes

NME7

NUT4

TNUTL

FCHLR

FCMFENT4/FCMFEC4

Note: DCLS Group Codes

NME7 PP

NTNP FCHLR

PNC FCMFENT4/FCMFEC4

Water Filtration for Genetic Molecular Analyses (VIMS)

1. Take water sample in water collection bottle. If unable to filter at site, place collection bottle on ice.
2. Attach funnel adapter and stopper to disposable test filter funnel. Stopper may not be necessary if using a branched filter flask. Place funnel adapter into neck of flask and rest filter funnel on rim. Filter 100 mls of water, adding 20 mls at a time, if necessary, due to turbidity of water.
3. Use disposable forceps to remove 3 um nuclepore filter from filtration apparatus. Fold filter in half and then in half again using the forceps and place in microcentrifuge tube containing buffer solution. With forceps, push filter into bottom of tube so that it is submersed in the liquid. Dispose of test filter funnel, collection bottle, and forceps. Do not dispose of funnel adapter, or stopper.
4. Label microcentrifuge tube (site, date, mls) and store upright in box provided. Samples should remain at room temperature.
5. Deliver samples and field data sheet to the Dr. Reece's Laboratory as soon as possible at VIMS, Chesapeake Bay Hall -North 119. Kim Reece – 804-864-7407, Gail Scott or Bill Jones - 804-684-7235/7873

Water and Sediment Samples at Monitoring Stations to be used for Detecting the Presence of HABs (ODU)

1. Collect one Lugols preserved water sample (1000 ml) at the surface (<1m) using a hose connected to a pump, or with a collection (Alpha) bottle lowered to the lower depth.
2. Once per the monitoring season collect a grab sample of the superficial sediment layer with a Petit Ponar at each station. The top 2-4 cm should be transferred to a collection jar. No preservative is added. The sample should be kept in the shade, and not iced. Approximately 250 ml of sediment should be collected.
3. The Chain of Custody field data sheet and water and sediment samples should be delivered to the ODU Plankton Analysis Laboratory. Laboratory contacts are: Dr. Harold Marshall (757-683-4204), and the laboratory manager Todd Egerton (757-683-4994).

PERSONAL SAFETY

Virginia agencies will continue to use current protocols for personal safety related to routine activities in estuarine waters. This includes VDH's shellfish sampling program, DEQ's ambient water quality monitoring program and Chesapeake Bay monitoring program, VIMS's finfish survey activities, VMRC's enforcement activities and ODU's phytoplankton and zooplankton activities. DEQ and VIMS staff involved in HAB monitoring will use reasonable protection to avoid contact with the water, including Neoprene gloves.

RESPONSE TRAINING

All DEQ staff, prior to their participation in the agency HAB response program, will be able to demonstrate competency in collection, preservation and shipment of water and sediment samples for use in laboratory identification of HABs.

QA/QC

PRO and TRO monitoring staff will obtain field replicates and blanks of all samples at one station per month, to assure 10 percent QA/QC sampling. The samples will be labeled S1, S2 and EB in the Comprehensive Environmental Data System (CEDS).

DATA MANAGEMENT

The DEQ has the responsibility for data management for this grant. Wherever possible the DEQ CEDS mainframe application system will be used to manage and store environmental data. This requires DEQ field staff to routinely enter field data and the Division of Consolidated Laboratory Services (DCLS) to weekly transfer the additional analytical data electronically to the CEDS application.

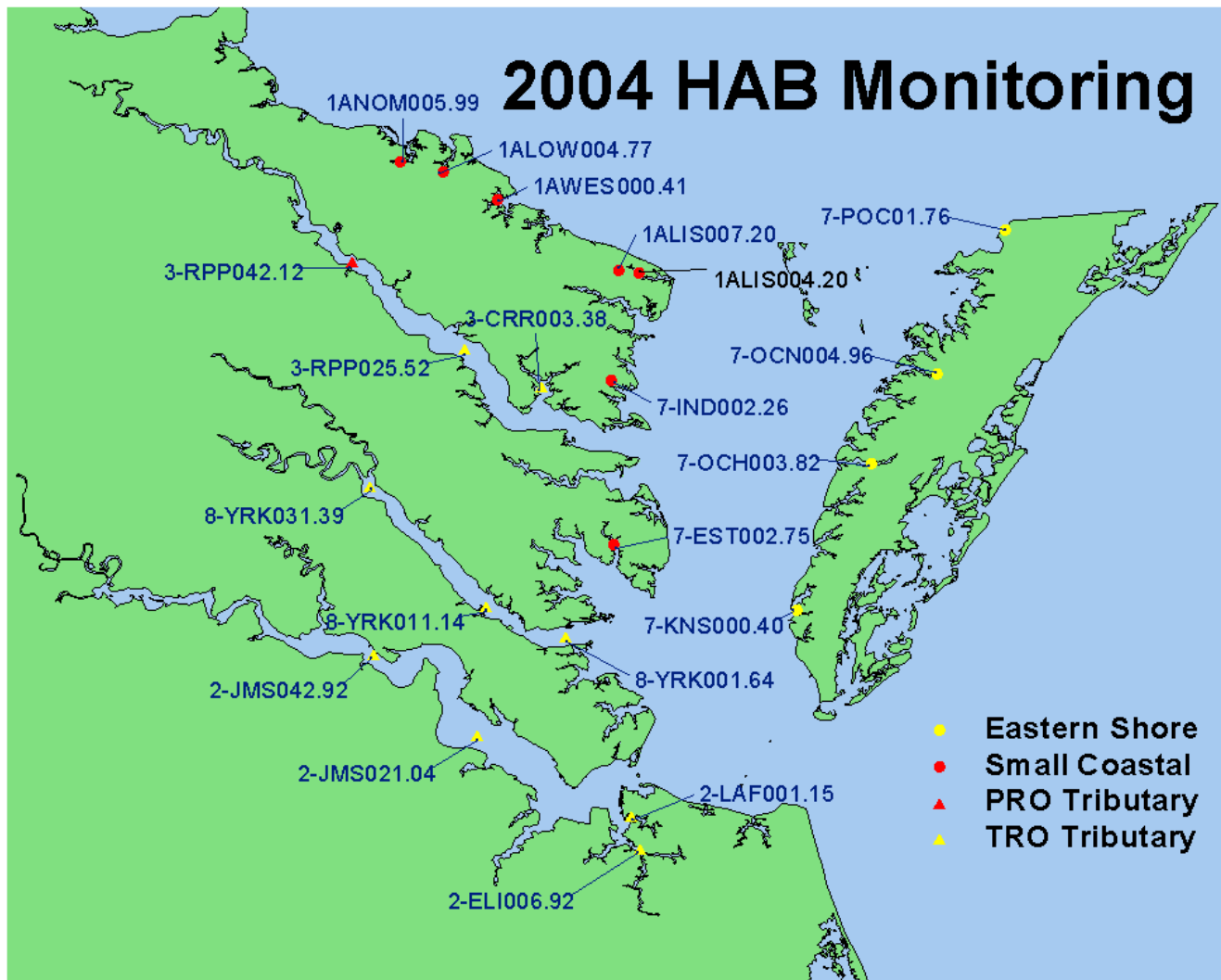
Data for quality assurance samples and samples shipped to other contract laboratories not on the Agency's CEDS system must be managed on field data sheets and lab sheets. DEQ will maintain a copy of all data sheets.

2004 HAB Monitoring Stations

VDH/VIMS/DEQ

REGION	RIVER BASIN	RIVER	STATION_ID	LOCATION	LAT_DD	LONG_DD
TRO	Rappahannock	Corrotoman River	3-CRR003.38	Red Buoy #6 (LE 3.3)	37.68842	-76.47442
TRO	Rappahannock	Rappahannock River	3-RPP025.52	Buoy #11, below Belle Is. (LE 3.1)	37.75925	-76.61592
TRO	James	James River	2-JMS021.04	Buoy C12-13 (LE 5.2)	37.05600	-76.59306
TRO	James	James River	2-JMS042.92	Swanns Point (RET 5.2)	37.20294	-76.78219
TRO	James	Elizabeth River	2-ELI006.92	Off Nauticus	36.84833	-76.29806
TRO	James	Lafayette River	2-LAF001.15	Hermitage Point (Buoy 10)	36.90833	-76.31460
TRO	York	York River	8-YRK001.64	Between Amoco Pier/Buoy 24 (LE 4.3)	37.23392	-76.43094
TRO	York	York River	8-YRK011.14	Buoy N34 (LE 4.2)	37.29044	-76.57811
TRO	York	York River	8-YRK031.39	Buoy C57 (RET 4.3)	37.50869	-76.78889
TRO	Small Coastal	Pocomoke	7-POC01.76	Rt. 709 Ramp	37.97488	-75.63180
TRO	Small Coastal	Onancock Creek	7-ONC004.96	Ramp	37.71181	-75.75608
TRO	Small Coastal	Occohannock Creek	7-OCH003.82	Rt. 606, Morley's Wharf	37.55028	-75.87639
TRO	Small Coastal	Kings Creek	7-KNS000.40	End of Rt. 680	37.28278	-76.01111
PRO	Potomac	Nomini Creek	1ANOM005.99	Rt. 202 Bridge	38.09944	-76.73361
PRO	Potomac	Lower Machodoc Creek	1ALOW004.77	Rt. 618	38.08028	-76.65528
PRO	Potomac	West Branch Yeocomic	1AWES000.41	Kinsale Marina	38.03039	-76.55692
PRO	Potomac	Little Wicomico Rive	1ALIS007.20	Rt. 646	37.90222	-76.33694
PRO	Potomac	Little Wicomico Rive	1ALIS004.20	Off Mouth of Spences Creek	37.89778	-76.30138
PRO	Small Coastal	Indian Creek	7-IND002.26	Kilmarnock Wharf	37.70056	-76.35000
PRO	Small Coastal	East River	7-EST002.75	Rt. 641, Williams Wharf	37.40333	-76.34750
PRO	Rappahannock	Rappahannock River	3-RPP042.12	Buoy R10	37.91730	-76.82220

- Station discontinued in June 2004; 1ALIS004.20 substituted



VDH –DSS

November 1993
Marine Biotoxins Memo

MEMORANDUM

DATE: November 12, 1993

TO: Division of Shellfish Sanitation Staff

FROM: Robert E. Croonenberghs, Ph.D., Director
Division of Shellfish Sanitation

THROUGH: Eric H. Bartsch, P.E., Director
Office of Water Programs

SUBJECT: Seawater Monitoring Program - Procedure - Marine Biotoxins

PURPOSE

The objectives of this policy are to:

1. Provide a procedure for an early warning system.
2. Provide a procedure to define the extent and severity of the occurrence.
3. Provide a procedure for effective State response to minimize illness.
4. Provide a procedure to ensure an adequate investigation, and that information is gathered and evaluated by qualified individuals.
5. Provide a procedure for closing and reopening contaminated areas in accordance with the National Shellfish Sanitation Program Manual of Operations.

EARLY WARNING SYSTEM

Gymnodinium breve (*Ptychodiscus brevis*) blooms originate in the eastern Gulf of Mexico. These blooms are not sudden population explosions but are normal population increases that are confined or physically concentrated by boundary layers, frontal systems, or convection cells sometimes in conjunction with the organisms vertical migratory behavior. In order to have an accumulation of organisms, it is only necessary to have conditions which favor the growth and dominance of a moderately large population of G. breve and the proper hydrographic and meteorological conditions. Blooms are then transported to nearshore waters by currents, tides and wind. Offshore G. breve blooms are sometimes detected as a red to brownish discoloration of surface waters or as massive fish kills.

The states of Florida and North Carolina have experienced significant problems with toxic shellfish poisonings due to G. breve blooms. Both of these states encounter significant Gulf Stream current influence. The influence of the Gulf Stream on Virginia inshore waters is normally insignificant. G. breve has a requirement for high salinity (>24 ppt), and so it is not frequently found in Virginia where the majority of clam and oyster resources exist. The North Carolina shellfish control agency will notify the Division of Shellfish Sanitation in the State of Virginia in the event that they detect significant levels of G. breve in their nearshore waters through their detection and monitoring program. When notified by North Carolina of a toxic bloom event, DSS will evaluate the Gulf stream current patterns as determined by satellite imagery and meteorological events. Predictable transport, nearshore water discoloration, nearshore fish kills or human respiratory irritation will initiate the sampling of the area in question.

In cases of other toxin producing algae the Division will rely upon the Department of Environmental Quality, Marine Resources Commission and other state agencies to alert the Division in the event of a bloom. At this point the bloom event sampling protocol described below will take place in conjunction with Dr. Marshall's lab and the notifying agency.

PROCEDURE

Routine Sampling

Monthly samples will be collected and identified by Dr. Harold Marshall at Old Dominion University and the presence of toxic marine algae reported to the Division of Shellfish Sanitation (DSS) at predetermined sampling stations throughout the bay. ROUTINE MONTHLY SAMPLING WILL BE DONE BY DR. MARSHALL.

DSS Sampling Responsibility

Bloom Event Sampling

Bloom event sampling will be conducted by DSS personnel in conjunction with Dr Harold Marshall's phytoplankton program at Old Dominion University. During the routine bacteriological water sampling that the DSS performs we will be alert to the presence of algae blooms. Upon discovery of these blooms DSS field personnel will be responsible for collecting samples. SPECIAL SAMPLING OF BLOOM EVENTS OF WHICH THE AGENCY IS AWARE WILL BE DONE BY DSS. If notified by other agencies, the Division will coordinate the collection and examination of the samples. All samples will be collected in prepared 250 ml or 500 ml nalgene bottles containing Lugol's preservative solution. These sample bottles will be carried on all sampling runs. At least five sample bottles will be carried at all times. The bloom should be sampled as follows:

1. In the event of an algal bloom or a fish kill, surface (upper 1 meter) water samples should be taken on site as soon as possible.

2. If the area of the bloom is less than 100 meters in length or width, then one sample shall be taken from the area that appears to have the highest concentration of organisms. This can be estimated by the intensity of water discoloration, i.e. sample from the portion of the bloom where the discoloration is most intense.
3. If the bloom is larger than 100 meters in length or width and it is the only bloom in the area, two samples shall be taken. Again, these samples shall come from the areas with the most intense discoloration.
4. If there are multiple blooms in the area of sampling, take one sample from a bloom in the approximate middle of the area containing the blooms and four from the blooms at equal distances along the perimeter of the area. Again sample the area within each of the five sample areas that has the most intense discoloration.
5. The samples should be tightly sealed and brought back to the field office. The field staff shall make arrangements in conjunction with the field director to have these samples transported to Dr. Marshall's laboratory at Old Dominion University so that they arrive within 48 hours of the time they were taken.
6. As soon as is feasible, Dr. Marshall will report the results to the central office, who will in turn relay this information immediately to the field office.
7. The following protocol for sample preparation has been provided by Dr. Marshal:

SAMPLING DURING ALGAL BLOOMS

- a. Any well rinsed plastic or glass container can be used. A 500 ml sample is adequate. If you have Lugol's preservative, add about 4 ml/500 ml sample, i.e. 2 ml per 250 ml bottle. No refrigeration of sample is necessary. The sample may be taken with a collection bucket, water sampler, or directly from the water.

- b. Be sure to record on the bottle label the date, time, location, plus the water temperature and salinity if possible.
- c. The water sample should be delivered to:
H. G. Marshall, Mills Godwin Life Science Bldg., Room
114, at Old Dominion University.

The mailing address is:

H. G. Marshall, Department of Biological Sciences
Old Dominion University, Norfolk, VA 23529-0266.

- d. In the event of a fish kill or an extensive bloom, please notify the Phytoplankton Laboratory at Old Dominion University at 804-683-4994, or leave a message for H. Marshall with the departmental secretary at 804-683-3595.
8. Most algal blooms in Virginia's tidal waters will be caused by dinoflagellates. The vast majority of these are nontoxin producing species, with associated fish kills caused by reduced oxygen conditions.

However, there are also a few toxin producing dinoflagellates in Virginia tidal waters, and these may also produce fish kills.

Recently, a dinoflagellate (Pfiesteria piscimorte) called the phantom dinoflagellate, has been associated with major fish kills in the Neuse and Pamlico estuaries in North Carolina. During the summer of 1992, this species was also reported from Jenkins Creek on the Choptank River in Maryland. There is special concern about the species because it produces large fish kills and its dynamic life cycle makes it difficult to associate with the kill since it quickly settles in the substrate. When this dinoflagellate produces a fish kill, the fish are known to appear disoriented and move suddenly or erratically. They may also have lesions on the body, especially near the mouth. The waters are also noticeably discolored.

If you suspect a P. piscimorte bloom, special care should be taken in taking water samples and to avoid contact with sensitive parts of the body. Unpreserved water should not be brought to other waters, or locations. Also, if you suspect this kind of fish kill, with these conditions, be sure to take water samples and inform the ODU Phytoplankton Laboratory as soon as possible.

Defining Severity and Extent of Bloom Events

When elevated concentrations (>1000 cells/liter) of G. breve are found, the initial bloom event sampling program shall be continued and expanded. In conjunction with Dr. Marshall at the ODU phytoplankton laboratory, sampling shall be conducted at predetermined stations in order to monitor the increase or decrease in cell concentrations. Sampling results, field reports, and hydrographic and meteorological data shall be evaluated by the classification chief to determine the severity and extent of a toxic algae bloom. If other toxic algae bloom occur, the Division will consult with Dr. Harold Marshall, ODU, and Dr. Sherwood Hall, FDA, to determine an appropriate course of action.

Restricting Harvesting

When G. breve cell concentrations equal or exceed 5,000 cells/liter in the creeks, bays, estuaries, or inlets, the adjacent estuarine shellfish harvesting areas shall be temporarily closed to harvesting. Evaluation of hydrographic and meteorological factors and water samples shall be used to determine the distribution of a bloom. If it is determined that additional shellfish harvesting areas will be impacted, those waters shall be temporarily closed. If other toxic algae bloom occur, the Division will consult with Dr. Harold Marshall, ODU, and Dr. Sherwood Hall, FDA, to determine an appropriate course of action.

Disposition of Product Harvested from Toxic Algae Bloom Areas

With an early warning system that includes timely and representative sampling, and prompt shellfish harvesting area closures, the harvest and distribution of potentially contaminated shellfish is unlikely. However, the Division of Shellfish Sanitation has the authority to examine shellfish records and requires all certified shellfish dealers to maintain adequate records in order to determine the distribution of potentially contaminated product and shall recall or embargo any such product.

Reopening of Harvest Areas Closed Due to Toxic Algae Blooms

Following area closure, water samples are collected at key, representative sampling stations, and G. breve cell counts determined. Once cell counts return to background levels, shellfish will be gathered for toxicity analysis. Shellfish shall be collected for toxicity analysis at sampling stations where shellfish are most likely to have been impacted. Areas closed to harvesting because of presence of G. breve shall not be reopened until counts are below 5,000 cells per liter inshore and offshore of the affected shellfish harvesting area, and shellfish meats have been shown to be free of toxin by laboratory analysis. If other toxic algae bloom occur, the Division will consult with Dr. Harold Marshall, ODU and Dr. Sherwood Hall, FDA to determine an appropriate course of action.

Procedures to Disseminate Information Concerning Toxic Algae Blooms

An important component in preventing shellfish poisoning is notifying the public and the industry of the danger associated with the harvesting and consumption of molluscan shellfish from areas where toxic algae blooms have occurred. Additionally, local health departments and health agencies in other states may need to be notified if potentially contaminated product makes its way into the distribution system. The Division shall notify these agencies as follows:

Industry - Direct notification of harvesters within affected areas by the Virginia Marine Resources Commission and processors by the Division of Shellfish Sanitation.

Local Health Department and other Health Agencies - Direct notification and written notification.

Public - Department of Health Public Information Officer will notify and educate the media and the public.

VDH –DSS


April 2004

Sampling for Warm Weather Phytoplankton
Memo

MEMORANDUM

DATE: April 28, 2004

TO: Division of Shellfish Sanitation Staff

FROM: Robert E. Croonenberghs, PhD, Director
Division of Shellfish Sanitation 

SUBJECT: Seawater Monitoring Program - Sampling for Warm Weather Phytoplankton

Delete Working Memo S-299

Purpose

The objectives of this protocol are:

1. To establish the effective dates of this protocol, which are from February 1 through October 31 each year until terminated. Sampling under this protocol begins February 1 for growing areas 1A through 13. Sampling for all other growing areas begins April 1.
2. To establish a uniform methodology for the collection of seawater and sediment samples to monitor phytoplankton populations **not as part of a bloom or red tide condition** (WM # S-244) and **not involved in a fish kill** (protective gear required - DEQ's responsibility).
3. To facilitate the collection and shipment of the samples to Dr. Harold Marshall at ODU.

Introduction

The Division is part of the HAB (Harmful Algal Bloom) Task Force and is continuing the effort being made to try to understand the ecology and potential public health effects of *Pfiesteria piscicida*, other related dinoflagellate algae, and harmful algal blooms in general. Dr. Harold Marshall, at Old Dominion University, has been studying the presence of phytoplankton species in the Chesapeake Bay for years. He analyzes our phytoplankton bloom samples for the presence of potentially toxic species.

The sampling effort described in this memo is designed to provide supportive information to the Estuary Associated Syndrome (EAS) cohort study conducted by the Office of Epidemiology in VDH. Both VIMS and DEQ will be providing specific monitoring information at sites designated in the cohort study. Samples collected by DSS under this working memo will be targeted to aid the cohort study and to provide information about other toxic phytoplankton species potentially present in shellfish growing waters.

Virginia tributaries to the Potomac River experienced elevated concentrations of the dinoflagellate *Dinophysis acuminata* in February 2002. This alga can and did produce detectable concentrations of okadaic acid, which can be responsible for diarrhetic shellfish poisoning (DSP) when in sufficiently high concentrations. Detectable concentrations of okadaic acid were found in oysters from the Potomac River, though the concentrations at that time were below those generally considered to be a risk for DSP to humans.

Other harmful algal blooms of concern include certain species of diatoms that could potentially produce domoic acid, which can cause amnesiac shellfish poisoning. Our February and March sampling in the Potomac tributaries will enable Dr. Marshall to better monitor for these species too.

Phytoplankton Sampling Design

1. Sampling conducted under this procedure is to be done in addition to any special sampling that might be done for red tide blooms (WM # S-244). If you see a red tide bloom, take samples in the bloom and a sample from the station indicated in this working memo. Dr. Marshall is very interested in these blooms.
2. The purpose of this sampling procedure is to provide information on a routine basis about the various species of phytoplankton present in shallow water areas of Virginia's portion of the Chesapeake Bay during the year from February 1 through October 31.
3. One phytoplankton sample shall be taken from approximately one half of the DSS seawater survey areas each month. The sampling locations are shown on the attached station list.
4. One sediment sample per station on the list will be collected once during the summer months; the timing is to be at the field director's discretion.
5. Whenever possible, samples should be taken in the more shallow waters adjacent to the station location, closer to the shoreline than in mid-channel. The exact sampling location is not critical because we are using the sample to reflect conditions for a much larger area than for our microbiological stations.
6. Samples should be delivered to Dr. Marshall generally at a maximum of every three weeks, and choice of the best delivery times is up to the field director. Our aim should be to avoid delivering several weeks' samples from all 3 field offices to Dr. Marshall at the same time.
7. All samples should be delivered to the Phytoplankton Lab (room 114, Life Science Bldg., Old Dominion University) week days, between 8:00am and 5:00pm. Phone: 757-683-4994.

Phytoplankton Sampling Procedure

1. Use pint size (473 ml) Nalgene sampling bottles to collect and store the samples. Label the bottles when dry with a Sharpie Brand (black is most durable) permanent marker as: DSS Phytoplankton Sample; growing area number-station number; date; water temperature, salinity, dissolved oxygen (DO) and turbidity.
2. Temperature, salinity, dissolved oxygen (DO) and turbidity shall be measured at the phytoplankton and sediment sampling sites.
 - Measure at the same depth as the water sample
 - Measure just above (not into) the bottom for the sediment sample.
 - DO rapidly decreases to zero within the very top few mm of most bottom sediments, and we want to avoid false zero readings.
 - Any zero DO readings should be confirmed by bringing the probe back to the surface and checking to be sure it is not covered with sediment, then carefully lowering back into the water.
 - DO readings are a key differentiator between unusual fish activity situations where there is sufficient oxygen for fish respiration (*i.e.*, potential *Pfiesteria* toxin) and where there is not enough oxygen (common anoxic fish kill situation). Be careful as this data may be widely and critically cited.
3. Sample water below the surface at elbow depth.
4. Add enough Lugol's solution to turn the water sample into a medium amber color after swirling the sample.
 - The amount needed will vary depending on the strength of the Lugol's solution.
 - Lugol's solution can be added to the sample container prior to leaving the office once you learn how much is needed for the strength of Lugol's mixture you use.
5. **Sediment samples are not to be preserved with Lugol's.** These samples are for growing out the live phytoplankton cysts at a later date.
 - Sample the bottom sediment with the 6"X6" (bite area) Ponar grab, take the sample from nearby shallower water if the station is located in over 10' of water, note approximate station location on the bottle.
 - Open the hatch on top of the Ponar grab and scoop off only the top 2 or 3 cm (1 inch) of surface sediment with a clean sampling spoon and place in the bottle.
 - Just less than half a bottle of sediment (200-250 grams) is sufficient.
6. Samples should be protected from high heat and extended bright light. These sediment and preserved water samples (as opposed to unpreserved phytoplankton water samples collected for blooms) may be transported on ice and stored in refrigeration. Protection from bright sunlight is the major concern here.

Phytoplankton and Sediment Sampling Sites

<u>Area</u>	<u>Site</u>	<u>Station Number</u>
1A	Upper Machodoc Creek	6
2	Monroe and Mattox Creeks	8, 18
4	Nomini River, Currioman Bay	10
5	Lower Machodoc Creek	16
6	Jackson Creek	4
7	Yeocomico River	12, 21, 42
8	Coan River, Glebe Creek	14, 32
9	Cod Creek, Cubitt Creek	2, 20
10	Little Wicomico River	6
12	Cockrell Creek	6
13	Great Wicomico River	3, 10, 23
15	Dividing Creek	11
20	Carter Creek	14
23	Deep Creek	2
25A	Rappa. River: Totusky Cr. to Tapp. Br.	14
28	Lagrange Creek	11
29	Urbanna Creek	3
33	Jackson Creek	13
34	Piankatank River	12
37	Stutts Creek	25
46	Perrin River	12
47	York River: Timberneck Creek	4
53	Poquoson River	18
58	Warwick River	10
61	Pagan River	7
62	Chuckatuck River	5
63	Nansemond River	3
65	W. Br. Elizabeth River	ER2
70	Lynnhaven River	9
71	Broad Bay	4
75	Pocomoke Sound	C6
76	Messongo Creek	5
80	Onancock Creek	8
81	Pungoteague Creek	3
82	Nandua Creek	3
83	Craddock Creek	5
85	Nassawadox Creek	8
92	Fisherman Island Inlet	1
93	Magothy Bay	6
95	Red Bank Creek	3
97	Folly Creek	18
98	Metomkin Bay	2
99	Island Hole Narrows	13
100	Mosquito Creek	5